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Metal Concentrations in Deep-Sea Mussels from a Hydrothermal Vent and Cold Seep

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METAL CONCENTRATIONS IN DEEP-SEA MUSSELS FROM A HYDROTHERMAL VENT AND COLD SEEP

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ABSTRACT

Concentrations of metals (Ca, Mg, Mn, Co, Cu, Zn, Cd) were described in tissues of the chemosynthetic mussels *Bathymodiolus thermophilus* collected directly from a hydrothermal vent on the East Pacific Rise (9°45'N, 104°17'W, 2500 m) and *B. heckerae* collected from a methane seep at Blake Ridge (32°31'N, 76°12'W, 2170 m). Short transplant experiments, where mussels were moved into the non-chemosynthetic deep sea, were also conducted at each site. Separate linear regression analyses based on metal storage in the form of granules and characteristics of the metal ions were used to explore the forms in which metals are stored in vent and seep mussels. Of the metals studied, Co concentrations were the lowest and Ca and Mg concentrations the highest in the gill, mantle, and visceral mass of vent and seep mussels. Metal concentrations were typically greatest in the gills and lowest in the mantle, with the visceral mass containing intermediate concentrations. The most apparent difference between metal concentrations in the tissue of mussels from the vent and seep was higher Cu concentrations in the gill, mantle, and visceral mass of vent mussels relative to seep mussels. Concentrations of metals in tissues from the vent and seep mussels were generally similar to metal concentrations in coastal mussels. The transplant experiments indicate that chemosynthetic mussels do not rapidly eliminate metals. Intermetal trends in metal concentrations for chemosynthetic mussels were correlated with χ^2r and softness index, suggesting ion characteristics may be useful in predicting metal concentrations in chemosynthetic mussels.

METAL CONCENTRATIONS IN DEEP-SEA MUSSELS FROM A HYDROTHERMAL VENT AND COLD SEEP

INTRODUCTION

Hydrothermal vents are naturally elevated in metals relative to other oceanic environments (Edmond and Von Damm 1985; Von Damm et al. 1985). Vent fluids are produced from cold seawater that seeps into the seafloor through cracks and fissures in the Earth's crust. Once inside the crust, the seawater is stripped of Mg and heated to greater than 300°C. Other elements are introduced into the fluid during interaction with molten rock before buoyant fluid re-enters the ocean (Edmond et al. 1982; Bonatti 1983). Hydrothermal vents often fuel chemosynthetic communities (Hessler and Smithey 1983), which are exposed to elevated temperatures and potential toxicants, including reduced compounds and heavy metals. Biochemical mechanisms for tolerating sulfides, including inactivation of sulfide by binding to sulfide-binding proteins in the vesicomyid clam *Calyptogena magnifica* and the vestimenteferan tubeworm *Riftia pachyptilla*, have been identified in organisms inhabiting hydrothermal vents (Powell and Somero 1986). To date, no novel mechanisms for metal tolerance have been identified in hydrothermal-vent organisms.

Cold seeps fuel chemosynthetic communities taxonomically similar to those found at hydrothermal vents. Organisms at cold seeps are exposed to reduced compounds, including methane and sulfide (Hecker 1985; Sibuet and Olu 1998). Reduced compounds enter the seeping fluid as a result of thermogenic or biogenic reactions within the ocean floor (Sibuet and Olu 1998). Unlike fluid from hydrothermal vents, reducing fluids from cold seeps are not characteristically elevated in metals. Taxonomic similarity between vent and seep fauna (Paull et al

1984; Sibuet and Olu 1998) allows for comparisons of related species that are adapted to environments that naturally vary in metal concentration. As a result of the differences in metal concentrations between these two habitats, mussel species found at vents may have evolved different mechanisms for metal regulation than mussels found at cold seeps.

Detrimental effects of metals from anthropogenic sources entering coastal ecosystems include carcinogenesis and interference with neural function (Anastassopoulou and Theophanides 2002; Gopal 2003). Programs such as NOAA's Mussel Watch have been established in order to monitor coastal pollutants, including metals (Goldberg et al. 1978). Mussels are useful as biomonitors due to their ability to accumulate pollutants in higher concentrations than that of their environment (Farrington et al. 1983). The sedentary adult lifestyle of mussels means that their metal concentrations tend to reflect the history of the environment from which they are collected (Farrington et al. 1983). The Mussel Watch program provides a large data set of metal concentrations in coastal mussels that can be compared with metal concentrations in mussels from other locales. It is possible that endemism to chemosynthetic habitats (Nelson and Fisher 1995) has allowed vent and seep mussels to evolve different mechanisms than their coastal counterparts for dealing with metal inundation. In the present study, metal concentrations in chemosynthetic mussels were compared with data from NOAA's Mussel Watch, in order to investigate metal concentrations in mussels from different habitats.

Metal toxicity can vary based on the particular metal, total concentration of bioavailable metal in the environment, and the organism itself (Rainbow 2002).

Many invertebrates bind metals with proteins (Roesidaji 1992; Roesidaji 1996) or sequester them in granules (Brown 1982). These detoxification mechanisms allow metals to remain metabolically available without exerting toxic effects on the organism. In coastal invertebrates, proteins (Geret and Cosson 2002) and granules (Klerks and Bartholomew 1991) are inducible in the presence of elevated metal concentrations. Both metallothioneins and granules have been noted in chemosynthetic organisms (Cosson and Vivier 1997; Geret et al. 1998; Rousse et al. 1998; Jenkins et al. 2002), but the extent to which each regulates metal concentrations within these mussels is unknown.

Mathematical models have been used to describe metal accumulation in shallow-water mussels based on either their affinity for granules or ion characteristics. Jeffree (1988) developed a theoretical model that predicts alkaline-earth metal storage in mussels that primarily accumulate metals in granules. The model predicts metal concentrations based on their competition with Ca for a binding site in the granule and is quantified based on linear relationships between the solubility of the metal phosphates and the concentration of the metals. Markich et al. (2001) extended Jeffree's (1988) model to predict concentrations of divalent metals in mussels. Tests of the model in the freshwater bivalves *Hyridella depressa* and *Velesunio ambiguus* in the Hawkesbury-Nepean River of south-eastern Australia showed that Ca concentrations were useful in predicting the concentrations of other metals in mussel tissues (Markich et al. 2001).

Metal accumulation in phytoplankton has been related to the log of the first hydrolysis constant of the metal, $|\text{Log } K_{OH}|$ (Fisher 1986). The log of the first

hydrolysis constant represents the affinity of a metal ion for oxygen. In addition to $|\text{Log } K_{OH}|$, softness index and $\chi^2 r$ (χ: electronegativity, r: atomic radius) have been modeled as predictors of metal toxicity (Newman et al. 1998). These ion characteristics reflect the tendency of the metals to bind with S, N, and O ligands, commonly found in proteins. Softness index, a measure of ability of the metal to donate electrons, was the ion characteristic that most accurately explained the toxicity data (Newman et al. 1998). In the current study, the granule-dominated model proposed by Jeffree (1988) and the ion characteristics proposed by Newman et al. (1998) were tested for their applicability in predicting metal accumulation in chemosynthetic mussels.

Previous studies provide some information on metal concentrations in mussels from hydrothermal vents, with some suggesting that specific metal concentrations may be elevated relative to those in coastal mussels (Smith and Flegal 1989; Rousse et al. 1998; Martins et al. 2001), but definitive conclusions have not been reached. Metal accumulation in cold-seep biota has not been investigated.

In order to more thoroughly investigate heavy metal storage by *Bathymodiolus* spp. in chemosynthetic ecosystems, concentrations of metals in tissues of mussels collected directly from a vent and seep were compared. Calcium, Mn, Co, Cu, Zn, and Cd were analyzed because they are known to be elevated in vent fluid (Von Damm et al. 1983; Douville et al. 2002). Magnesium is of interest due to its absence in vent fluid (Von Damm et al. 1998). Short transplant experiments, where mussels were moved into the non-chemosynthetic deep sea, were conducted at each site to investigate the rates at which metals were excreted. Linear regression analyses based

on work by Markich et al. (2001) and Newman et al. (1998), were used to explore metal storage in vent and seep mussels

MATERIALS AND METHODS

Sample collection

Mytilid mussels were collected by DSV *Alvin* from two deep-sea chemosynthetic communities (Figure 1) for elemental analyses. *Bathymodiolus heckerae* were sampled in September 2001 on the Deep-East Expedition to Blake Ridge (32°31'N, 76°12'W, depth 2170 m), a methane seep off the South Carolina coast. A description of the Blake Ridge site can be found in Van Dover et al. (2003). Animals were collected from a mussel bed heavily populated with juveniles. *Bathymodiolus thermophilus* were collected from the East Wall hydrothermal vent site during a December 2001 cruise to 9°N (9°45'N, 104°17'W, depth 2500 m) on the East Pacific Rise. A site description of 9°N can be found in Shank et al. (1998). At the time of sampling, both tubeworms (*Riftia pachyptila*) and mussels (*B. thermophilus*) colonized sites of diffuse flow at East Wall.

Two types of samples were collected: mussels retrieved directly from the site and mussels transplanted away from the influence of vent or seep fluids. Directly collected mussels were placed into an acrylic box with metal hinges for transport to the surface. At Blake Ridge these mussels were collected with a metal and plastic mesh scoop, while mussels at 9°N were individually collected with *Alvin's* titanium manipulator. All mussels used in the transplant experiments were collected by *Alvin's* manipulator and placed into plastic milk crates. These crates were moved to

an area of no discernible flow 20 m from their collection site. Transplanted milk crates on Blake Ridge were collected during their 4th day of transplant. Those at 9°N were collected during their 7th or 12th days of transplant.

On deck, all mussels were immediately moved into a cold room (4°C). Fifteen mussels from each collection were stored in plastic bags and frozen to -70°C within an hour of surfacing. Samples were kept frozen during transport to the laboratory, where all samples were stored at -70°C until processing.

Laboratory analyses

In order to prepare samples for elemental analysis, the gill, mantle, and visceral mass were dissected separately from frozen mussels and freeze-dried. Shell lengths (±0.1 cm) were recorded for each mussel. The gill, mantle, and visceral mass from several mussels were divided in half during dissection to serve as replicates. Samples were ground to powder after freeze-drying for at least 48 hrs. Half a gram of each tissue was digested with 5 mL of Teflon®-distilled HNO_3 in a Microwave Accelerated Reaction System (MARS 5, CEM Corp., Matthews, NC). Following digestion, samples were brought up to 25 mL total volume with deionized water and stored in 50-mL, acid-cleaned high-density polyethylene bottles. One blank (deionized water and acid only) and two standard reference materials were run during each digestion. Reference materials used were SRM Oyster Tissue 1566a from the National Institute of Standards and Technology and DOLT-1 from the Marin Analytical Chemistry Standards Program in the Division of Chemistry of the National Research Council of Canada. Elemental analyses were carried out by atomic

absorption spectroscopy (AAS) with a Perkin-Elmer AAnalyst 800 (Perkin-Elmer, Shelton, CT). Magnesium, Ca, Mn, Cu, Zn, and Cd were analyzed by flame AAS. Graphite Furnace AAS was used to measure Co concentration. All materials in contact with the tissue in the laboratory were trace-metal clean, and standard reference materials yielded metal concentrations within the range of reference values.

Due to the small size of mussels collected directly from Blake Ridge, 2 of the seven samples analyzed from that collection consisted of 3 pooled mussels in each sample, and 2 samples each consisted of 4 pooled mussels. Tissues were combined immediately following dissection.

All metal concentrations were standardized to μg/g dry wt and expressed as mean ± standard deviation. Replicate samples were averaged. Two questionable data points were not included in the statistical analyses. One value for Ca concentration in the visceral mass of a vent mussel, *B. thermophilus,* was excluded because replicates of the sample differed by an order of magnitude (2942 μg/g, 46476 μg/g). In addition, one value for Ca concentration (33,288 μg/g) in the mantle of a seep mussel, *B. heckerae*, was excluded because it differed from all other samples by an order of magnitude and failed a statistical outlier test (Grubbs 1979).

Statistics

Two-sample t-tests were run with Minitab (Release 13.32) to evaluate differences in specific metal concentrations between site, transplant duration, and tissue type. A Bonferroni adjustment was used for 2-sample t-tests comparing metal concentrations between habitats and between transplant days ($\alpha_{pairwise} = 0.0024$). All

other significant statistical differences were determined with an $\alpha_{pairwise} = 0.05$. Simple linear regression analyses were used to evaluate metal storage in granules and proteins. Principal component analyses (PCA), carried out in Minitab, were used to compare metal storage in vent and seep mussels. Data for all measured metals was obtained for five mussels from each site. Only these mussels were included in the PCA.

Linear regressions were carried out in Minitab in order to identify relationships between metal concentration and: shell length, Ca concentration, metal phosphate solubility (K_{PO4}), $\chi^2 r$ (χ: electronegativity, r: Pauling ionic radius), softness index, and $|\log K_{OH}|$ (log of first hydrolysis constant of metal ion). Phosphate solubilities were obtained from Markich et al. (2001). Values for $\chi^2 r$, softness index, and $|\log K_{OH}|$ were tabulated by McCloskey et al. (1996).

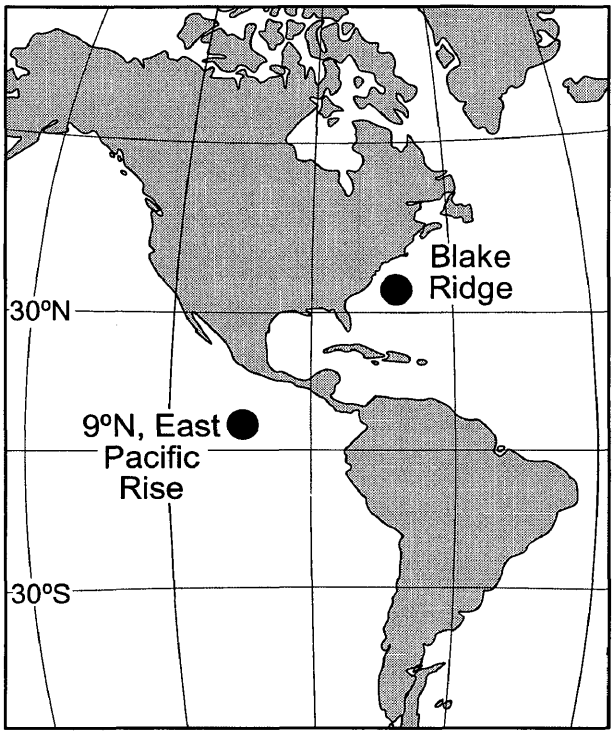

Figure 1

RESULTS

Trends within mussels collected directly from the habitat

Mussels analyzed from Blake Ridge cold seep were more variable in size than those from the hydrothermal vent at 9°N. Shell lengths of *Bathymodiolus heckerae* collected directly from the seep site ranged from 4.8 cm to 33.0 cm (mean = 12.5 ± 10.3 cm), and those of the vent mussel *B. thermophilus* ranged from 9.1 cm to 11.0 cm (mean = 9.9 ± 0.6 cm).

Cobalt concentrations in both *B. thermophilus* and *B. heckerae* were lower than those of all other metals in the gill, mantle, and visceral mass (Table 1). In both species, concentrations of Ca and Mg in the gill, mantle, and visceral mass were higher than all other metals, with Mg being higher in *B. thermophilus* and Ca being higher in *B. heckerae*.

In general, the highest concentrations of metals were found in the gills and lowest concentrations were found in the mantle (Table 1). Exceptions to this trend were significantly elevated Ca ($p = 0.038$) and Mg ($p = 0.036$) concentrations in the mantle of the vent mussel *B. thermophilus* relative to their concentrations in the visceral mass. Mussels from neither habitat exhibited metal concentrations that were significantly higher ($\alpha_{pairwise} = 0.05$) in the mantle or visceral mass than the gill.

Habitat comparison

In comparing mussels collected directly from the vent and seep habitats, the concentration of Cu was significantly higher ($p < 0.0005$) in the gill and visceral mass of the vent mussel *B. thermophilus* relative to the seep mussel *B. heckerae* (Table 1, Figure 2). No other metals were found to be significantly higher in mussels from the vent than the seep. The seep mussel *B. heckerae* had significantly higher concentrations of Cd in the mantle and visceral mass compared to *B. thermophilus* from the vent. Though not all differences were statistically significant, Ca, Cd, and Co concentrations were all higher in the gill, mantle, and visceral mass of the seep mussel than in the vent mussel.

Principal component analysis (PCA) supports the idea that metals were accumulated differently in the vent and seep mussels studied, and that much of this dissimilarity was due to differences in Cu accumulation (Table 2). When data from all 7 metals in all tissue types were analyzed together, principal component 1 (PC1), which assigned negative values to the seep mussels and positive values to the vent mussels, explained 47% of the variation in the data. Investigation of the gill, mantle and visceral mass individually led the percent variation explained by PC1 to rise to 51%, 53%, and 50% respectively. PCA results that incorporated all tissues yielded a PC1 that was positively correlated with concentrations of Zn in the gill and Cu in all three tissues, and was negatively correlated with Zn and Cd in the mantle and visceral mass and Ca and Co concentrations in all three tissues. Analysis of gill concentrations alone produced a PC1 negatively correlated with Cu and Zn concentrations and positively correlated with Ca and Co concentrations. Principal

component analysis of metal concentrations in the mantle generated a PC1 that was positively correlated with Cu and Mg concentrations and negatively correlated with Ca, Mn, Co, Zn, and Cd concentrations in the mantle. Analysis of only those metal concentrations in the visceral mass yielded a PC1 that was positively correlated with Cu concentration and negatively correlated with Co, Ca, Zn, and Cd concentrations. A contrary trend was found when only concentrations of metals in the gill were included, where positive PC1 values were assigned to vent mussels and negative PC1 values were assigned to seep mussels.

Transplanted mussels

No significant changes ($\alpha_{pairwise} = 0.0024$) in the concentrations of metals in the tissues of the cold seep mussel *B. heckerae* or the vent mussel *B. thermophilus* occurred during the transplant experiments (Tables 3 and 4) when compared to mussels collected directly from their respective chemosynthetic environments (Table 1). Nine measurements of tissue-specific metal concentrations decreased during the first 7 days of the transplant, while all but 5 increased between the 2^{nd} and 3^{rd} time points of the transplant experiment.

Linear regression analyses for predicting metal storage

Two ion characteristics, χ^2r and softness index, were significant predictors of metal concentrations in the gill, mantle, and visceral mass of mussels from both the vent and seep sites (Table 5). The one exception to this was softness index as a predictor for metal concentration in the gills of the vent mussel *Bathymodiolus*

thermophilus. The absolute value of log K_{OH} was found to be a significant predictor of metal concentrations in the gill, mantle, and visceral mass of the seep mussel *Bathymodiolus heckerae* (Table 5). Linear regressions evaluating dependence of metal concentrations in particular tissues on shell length suggest that shell length was correlated with accumulation of metals (Ca, Mn, Co, Zn, Cd) in the gills of the cold seep mussel *B. heckerae* (Table 6). Linear regressions evaluating the dependence of metal concentrations in particular tissues on the concentration of Ca within the same tissue (Table 7) and K_{PO4} (Table 5) did not indicate a significant correlation between these factors.

Table 1. Metal concentrations [mean μg/g dry wt., (±s.d.)] in *Bathymodiolus* species. n = 7 unless otherwise noted: [a]n = 6, [b]n = 5; g, m, or v indicates that the gill, mantle, or visceral mass, respectively, is significantly different ($p < 0.05$) than the tissue containing the designation; * indicates significant difference ($\alpha_{pairwise} = 0.0024$) between habitats

		B. thermophilus hydrothermal vent		*B. heckerae* cold seep		Habitat/Species Comparison p-value
Mg						
	gill	[a]$9061_{m,v}$	(1234.61)	$8630_{m,v}$	(1411.94)	0.555
	mantle	$6661_{g,v}$	(1645.94)	4892_{g}	(1258.34)	0.045
	visceral mass	$4825_{g,m}$	(1142.14)	4207_{g}	(1232.58)	0.351
Ca						
	gill	3612_{v}	(657.45)	9362_{v}	(3173.10)	0.003
	mantle	3137_{v}	(908.05)	8744	(11061.83)	0.23
	visceral mass	[a]$2150_{m,g}$	(529.33)	[a]4874_{g}	(2796.93)	0.045
Mn						
	gill	16.82_{m}	(9.52)	9.84	(4.45)	0.117
	mantle	6.57_{g}	(2.01)	6.38	(3.24)	0.898
	visceral mass	11.50	(5.60)	6.90	(2.43)	0.082
Co						
	gill	0.11	(0.15)	0.39_{m}	(0.17)	0.009
	mantle	[a]0.036_{v}	(0.05)	$0.11_{g,v}$	(0.11)	0.141
	visceral mass	0.14_{m}	(0.06)	0.26_{m}	(0.13)	0.068
Cu						
	gill	$178.45_{m,v}$	(29.21)	$94.14_{m,v}$	(19.47)	<0.0005*
	mantle	$21.60_{g,v}$	(7.82)	8.15_{g}	(4.74)	0.004
	visceral mass	$32.15_{g,m}$	(3.34)	8.98_{g}	(4.54)	<0.0005*
Zn						
	gill	$102.30_{m,v}$	(21.48)	[b]75.46	(12.52)	0.024
	mantle	$40.16_{g,v}$	(10.41)	[b]58.01	(11.39)	0.024
	visceral mass	$53.59_{g,m}$	(9.31)	[b]60.52	(4.86)	0.128
Cd						
	gill	[a]$5.13_{m,v}$	(0.63)	5.94_{m}	(0.94)	0.097
	mantle	[a]$0.33_{g,v}$	(0.27)	$1.12_{g,v}$	(0.37)	0.001*
	visceral mass	[a]$1.47_{g,m}$	(0.57)	10.44_{m}	(5.56)	0.005

Table 2. Principal Component Analysis (PCA) results. Columns contain PC1 eigenvectors for each analysis. A large eigenvector (+ or -) indicates that the metal in question contributes strongly to differences between vent and seep mussel concentrations. Empty spaces indicate data from variable was not used in analysis.

	all tissues	gill	mantle	visceral mass
Mg gill	-0.123	0.205		
Mg mantle	0.105		0.353	
Mg visceral mass	0.019			0.174
Ca gill	-0.309	0.493		
Ca mantle	-0.233		-0.338	
Ca visceral mass	-0.270			-0.419
Mn gill	0.093	-0.207		
Mn mantle	-0.086		-0.320	
Mn visceral mass	0.123			0.184
Co gill	-0.302	0.456		
Co mantle	-0.234		-0.390	
Co visceral mass	-0.286			-0.435
Cu gill	0.295	-0.509		
Cu mantle	0.233		0.448	
Cu visceral mass	0.310			0.516
Zn gill	0.227	-0.441		
Zn mantle	-0.224		-0.445	
Zn visceral mass	-0.190			-0.349
Cd gill	-0.013	0.100		
Cd mantle	-0.280		-0.328	
Cd visceral mass	-0.197			-0.427

Table 3. Metal concentrations [mean μg/g dry wt. (±s.d.)] in transplanted *Bathymodiolus heckerae*. n = 7unless otherwise noted: [a]n = 6, [b]n = 5 [c]n=3 [d]n=2; no significant difference ($\alpha_{pairwise}$ = 0.0024) between mussels collected directly from Blake Ridge cold seep and those transplanted away from seeping fluid for 4 days

		cold seep: day 4 *B. heckerae*		Transplant Comparison p-value
Mg				
	gill	8408	(2216)	0.827
	mantle	[a]2915	(2691)	0.15
	visceral mass	4305	(2935)	0.937
Ca				
	gill	3712	(1243)	0.003
	mantle	[a]1981	(2142)	0.164
	visceral mass	2277	(975)	0.053
Mn				
	gill	5.24	(2.31)	0.038
	mantle	[a]4.29	(1.97)	0.184
	visceral mass	5.30	(1.82)	0.188
Co				
	gill	0.27	(0.21)	0.26
	mantle	[a]0.03	(0.01)	0.11
	visceral mass	0.14	(0.07)	0.181
Cu				
	gill	77.76	(14.89)	0.105
	mantle	[a]5.73	(7.46)	0.512
	visceral mass	6.33	(5.46)	0.344
Zn				
	gill	[c]82.12	(5.50)	0.348
	mantle	[d]36.27	(11.39)	0.051
	visceral mass	[c]51.07	(4.86)	0.449
Cd				
	gill	6.85	(1.59)	0.225
	mantle	[a]0.82	(0.89)	0.483
	visceral mass	10.20	(7.44)	0.947

Table 4. Metal concentrations [mean μg/g dry wt. (±s.d.)] in transplanted *Bathymodiolus thermpohilus*. n = 7unless otherwise noted: [a]n = 6, [b]n = 8 [c]n=4; no significant differences ($\alpha_{pairwise}$ = 0.0024) were found between metal concentrations during the transplant experiment

		hydrothermal vent *B. thermpohilus* transplant day 7		hydrothermal vent *B. thermpohilus* transplant day 12		Transplant Comparison (Day 1 v Day 7) p-value	Transplant Comparison (Day 7 v Day 12) p-value
Mg							
	gill	[a]7170	(386)	[b]8184	(1368)	0.006	0.141
	mantle	6049	(1657)	[b]4928	(1318)	0.502	0.179
	visceral mass	3945	(991)	4348	(766)	0.152	0.082
Ca							
	gill	[a]2777	(251)	[b]3032	(639)	0.017	0.330
	mantle	2438	(600)	[b]1772	(790)	0.120	0.089
	visceral mass	1619	(452)	1624	(279)	0.085	0.980
Mn							
	gill	[a]7.36	(0.27)	[b]8.24	(1.54)	0.039	0.158
	mantle	5.50	(0.88)	[b]5.66	(1.03)	0.232	0.743
	visceral mass	9.17	(3.68)	10.90	(3.40)	0.381	0.382
Co							
	gill	0.09	(0.07)	[b]0.14	(0.06)	0.779	0.203
	mantle	0.05	(0.08)	[b]0.36	(0.36)	0.751	0.024
	visceral mass	0.12	(0.06)	0.17	(0.08)	0.575	0.244
Cu							
	gill	190.66	(13.85)	[b]179.43	(21.66)	0.347	0.249
	mantle	31.52	(27.41)	[b]47.09	(30.41)	0.392	0.318
	visceral mass	38.55	(30.41)	37.59	(5.25)	0.117	0.810
Zn							
	gill	[c]132.40	(18.88)	[b]157.98	(27.78)	0.023	0.065
	mantle	42.71	(7.02)	140.47	(63.49)	0.602	0.064
	visceral mass	57.72	(8.17)	74.37	(27.69)	0.397	0.171
Cd							
	gill	[c]5.57	(0.85)	[b]6.24	(0.88)	0.420	0.251
	mantle	[c]0.23	(0.15)	[b]0.90	(0.54)	0.468	0.011
	visceral mass	[c]1.48	(0.14)	1.91	(0.45)	0.977	0.050

Table 5. P-values for linear regressions where ion characteristics are modeled as predictors of metal concentrations in in the gill, mantle, and visceral mass (vm) of vent and seep mussels. *values are significant ($\alpha_{pairwise} = 0.05$)

Ion Characteristics	*B. thermophilus* hydrothermal vent			*B. heckerae* cold seep		
	gill	mantle	vm	gill	mantle	vm
Log K_{PO4}	0.139	0.145	0.143	0.213	0.329	0.228
X^2/r	0.049*	0.034*	0.038*	0.005*	0.008*	0.005*
Softness Index	0.062	0.045*	0.050*	0.008*	0.010*	0.007*
Log K_{OH}	0.126	0.093	0.103	0.020*	0.016*	0.018*

Table 6. P-values for linear regressions where shell length is a predictor of metal concentrations in the gill, mantle, and visceral mass (vm) of vent and seep mussels. *values are significant ($\alpha_{pairwise} = 0.05$)

Metal	*B. thermophilus* hydrothermal vent			*B. heckerae* cold seep		
	gill	mantle	vm	gill	mantle	vm
Mg	0.940	0.968	0.892	0.916	0.792	0.127
Ca	0.528	0.168	0.376	0.044*	0.284	0.315
Mn	0.520	0.932	0.165	0.004*	0.619	0.654
Co	0.288	0.534	0.140	0.011*	0.254	0.125
Cu	0.074	0.263	0.752	0.666	0.042*	0.308
Zn	0.763	0.828	0.390	0.019*	0.094	0.363
Cd	0.041*	0.312	0.321	0.012*	0.200	0.291

Table 7. P-values for linear regressions where [Ca] is a predictor of other metal concentrations in in the gill, mantle, and visceral mass (vm) of vent and seep mussels. *values are significant ($\alpha_{pairwise} = 0.05$)

Metal	*B. thermophilus* hydrothermal vent			*B. heckerae* cold seep		
	gill	mantle	vm	gill	mantle	vm
Mg	0.014*	0.086	0.019*	0.610	0.334	0.481
Mn	0.505	0.428	0.026*	0.014*	0.002*	0.540
Co	0.176	0.474	0.835	0.001*	<0.0005*	0.100
Cu	0.867	0.015*	0.645	0.368	0.905	0.720
Zn	0.584	0.154	0.018*	0.083	0.815	0.535
Cd	0.811	0.269	0.920	0.274	0.889	0.588

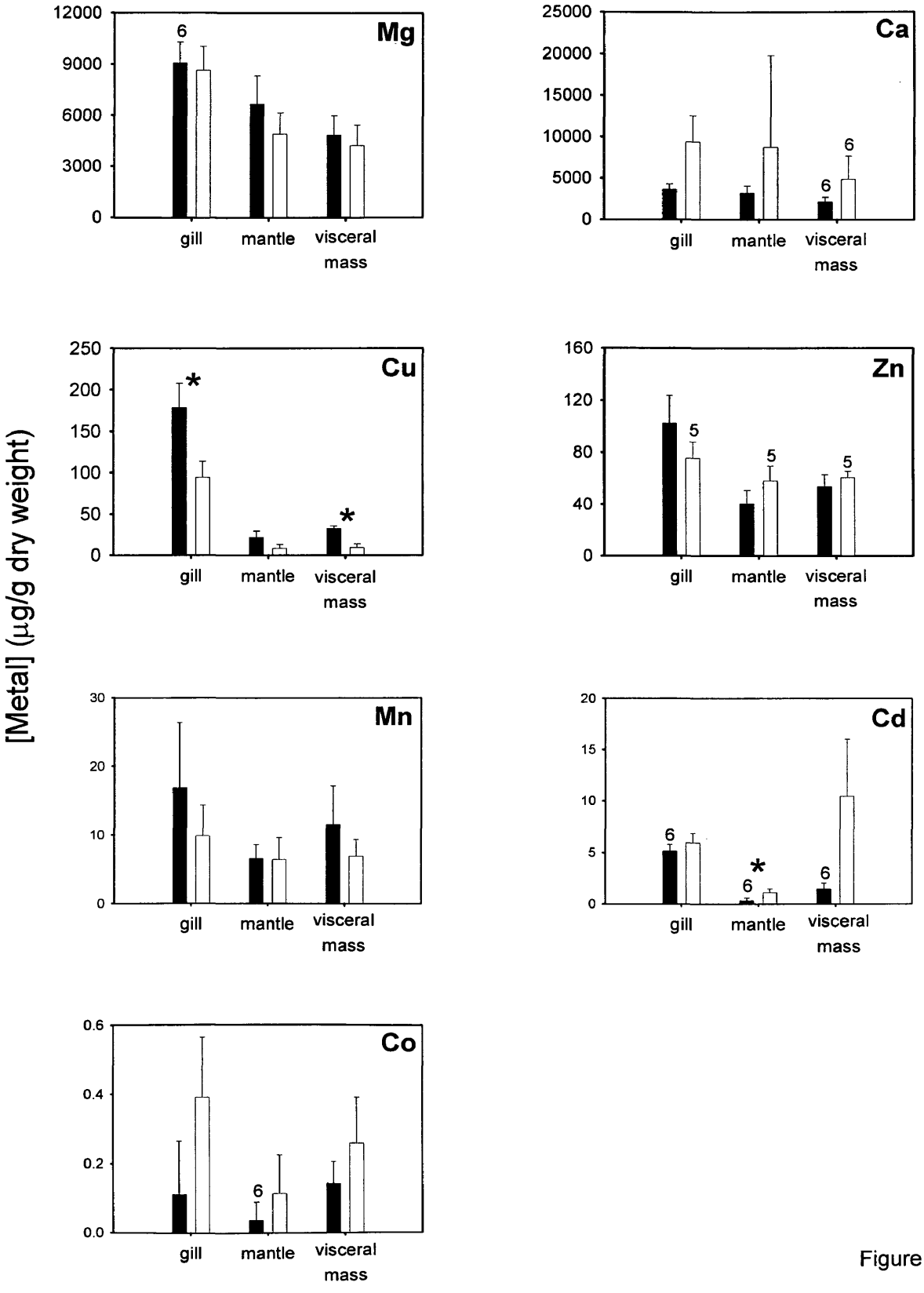
[Metal] (µg/g dry weight)
Mg
12000
9000
6000
3000
0
6
gill
mantle
visceral mass
Ca
25000
20000
15000
10000
5000
0
6
6
gill
mantle
visceral mass
Cu
250
200
150
100
50
0
*
*
gill
mantle
visceral mass
Zn
160
120
80
40
0
5
5
5
gill
mantle
visceral mass
Mn
30
20
10
0
gill
mantle
visceral mass
Cd
20
15
10
5
0
6
*
6
6
gill
mantle
visceral mass
Co
0.6
0.4
0.2
0.0
6
gill
mantle
visceral mass

Figure 2

DISCUSSION

Comparisons of Ca, Mg, Mn, Cu, Co, Cd, and Zn concentrations in mussels from hydrothermal vents and cold seeps showed that, in general, Co concentrations were lowest and Ca and Mg concentrations were highest. Metal concentrations tended to be greatest in the gills and lowest in the mantle, with the visceral mass containing intermediate concentrations. The most apparent difference between metal concentrations of *Bathymodiolus* spp. mussels from vents and seeps was higher copper concentrations in the gill, mantle, and visceral mass of vent mussels relative to seep mussels.

Lower concentrations of Co than Ca, Mg, Mn, Zn, Cu or Cd in the tissues of *Bathymodiolus thermophilus* from the hydrothermal vent at 9°N are consistent with measurements of whole body tissue in *B. thermophilus* from the Galapagos Spreading Center, as are higher concentrations of Ca and Mg than other metals studied (Smith and Flegal 1989). In the previous study, concentrations of Cl and Na were higher than concentrations of Ca and Mg; Cl and Na were not measured in the current study. Zinc, Cu, Mn, and Cd concentrations found in the current study were similar to those measured in *Bathymodiolus azoricus* from hydrothermal vents on the Mid-Atlantic Ridge (Rousse et al. 1998). High accumulations of Fe, Ag, Ba, and Sr were reported in gill and visceral mass of *B. azoricus* (Rousse et al. 1998); these metals were not measured in the current study.

Between vent sites, the chemistry of hydrothermal fluids varies due to specific chemical, thermal, and mineralogical conditions (Metz and Trefry 2000). The dynamic conditions at individual venting sites result in chemistry that varies over time (Johnson et al. 1988; Chevaldonne et al. 1991; Von Damm et al. 1997; Sarradin et al. 1999). The variation noted in the Hg levels of *B. azoricus* from three vent sites on the Mid-Atlantic Ridge reflects differences in chemistry of the venting fluids (Martins et al. 2001). While concentrations of trace metals in vent fluid surrounding the mussels were not quantified during this study, previous research indicates high concentrations of metals in fluid surrounding mussel beds (Sarradin et al. 1999).

Fluids from cold seeps have elevated methane and sulfide concentrations relative to the ambient deep sea (Sibuet and Olu 1998); but, unlike fluid from hydrothermal vents, seeping fluids are not thought to be characterized by elevated metal concentrations. Concentrations of metals in the seeping fluids and seawater at Blake Ridge have not yet been quantified; however, the Ocean Drilling Program measured metal concentrations in the sediment (Paull et al. 2000). In the top 21 cm of sediment, metals were present from highest to lowest concentrations as: [CaO] > [MgO] > [Zn] > [Cu] > [Co] > [Mn] > [Cd]. Highest concentrations of Ca relative to other metals in tissues of the seep mussel, *B. heckerae*, are consistent with elevated Ca concentrations relative to other metals in the sediment. Unlike the sediment data, Co rather than Cd has the lowest concentration in the tissues of the seep mussel.

The difference in chemistry between venting and seeping fluid might lead us to expect differences in metal accumulation between the two habitats. Two-sample t-tests and PCA both indicate that concentrations of Cu in the gill, mantle, and visceral

mass of the vent mussels were higher than their respective concentrations in the seep mussels. One possible factor that might explain significantly higher Cu concentrations in the tissues of vent mussels relative to the same tissues in seep mussels is greater bioavailability of Cu than other metals at vents. Vent mussels may also preferentially accumulate Cu relative to other metals. While the concentration of Cu in mussel tissues differs significantly between vent and seep habitats, concentrations of Mg, Ca, Mn, Co, and Zn were not significantly different in the gill, mantle, or visceral mass between mussels from the two habitats (Table 1).

Two factors confound the habitat comparison in the present study. First, while the two mussels studied were both of the genus *Bathymodiolus*, they were of different species. While *B. thermophilus* and *B. heckerae* are taxonomically similar and both are nutritionally reliant on bacterial symbionts, it is possible that any difference in metal regulation by these two mussels results from physiological differences between the two species. A second confounding factor was the difference in size of the mussels from the two habitats. Metal concentrations in coastal mussels, standardized by tissue weight, have been inversely related to shell length (Newman and Heagler 1991). The size discrepancy between the two species is evident in the maximum sizes of the hydrothermal-vent mussel *B. thermophilus* (11.0 cm) compared to the maximum size of the cold-seep mussel *B. heckerae* (33.0 cm) collected for this study. The difference in size between mussels from the two habitats may reflect a difference in age between the individual mussels. While hydrothermal vents tend to be ephemeral (Haymon et al. 1991), seeps are fairly long lived (Sibuet and Olu 1998). If mussels at Blake Ridge cold seep are older than those from the hydrothermal vent

at 9°N, then the seep mussels would have had a longer time over which to concentrate metals.

Transplant experiments were conducted to observe the effect of a low-metal environment on concentrations of metals in vent mussels. Lack of any significant changes in metal concentrations during the transplant experiments suggest that *Bathymodiolus* spp. does not rapidly eliminate metals. Increases in metal concentrations in mussel tissues were not expected during exposure to lower environmental metal concentrations relative to those mussels collected directly from the vent site. *Bathymodiolus* spp. have filter-feeding capabilities (Page et al. 1990; Page et al. 1991) but are generally reliant on bacterial symbionts in their gills for nutrition (Cavanaugh 1983; Rau 1985). These gill symbionts oxidize sulfide from the chemosynthetic fluid during the fixation of CO_2 (Cavanaugh 1983). Starvation of the mussels may have reduced the tissue weight, leading metals to concentrate at higher levels in the remaining tissue. While no gross differences were noted between the mussels collected after being transplanted and those collected directly from the vent, collections from the final transplant day at 9N were gaping more than mussels from other collections.

Copper concentrations in all tissues from vent mussels and the gills of seep mussels exceeded concentrations measured for the whole tissue of coastal Mytilids (Golberg et al. 1978; Goldberg et al.1983). Fifteenth, 50^{th}, and 85^{th} centile values were calculated from concentrations of metals measured in mussels from 214 sites by NOAA's Mussel Watch Program (O'Connor 2002). Concentrations greater than the 85^{th} centile value were considered "high" and those below the 15^{th} centile were

considered "low." The 85th centile concentration for Cu, 12 μg/g dry wt., was greatly exceeded by the mean concentrations of Cu in the gills of *Bathymodiolus thermophilus* (178 μg/g dry wt.) and *B. heckerae* (94 μg/g dry wt.).

The Cd concentration in the visceral mass of the seep mussels (10.44 μg/g dry wt.) was higher than the O'Connor's (2002) 85th centile concentration (5.9 μg/g dry wt.). This combined with significantly higher Cd concentrations in the mantle and visceral mass of the seep mussels relative to Cd concentrations in the vent mussels leads to questions about Cd in the Blake Ridge environment. It is possible that Cd in Blake Ridge waters may be elevated relative to unpolluted coastal environments. Based on the observation that the concentration of Cd in the gills of seep mussels is not elevated but both mantle and visceral mass are elevated relative to vent mussels, it is also possible that the seep mussel *B. heckerae* preferentially accumulates Cd.

Aside from Cu concentrations in the vent mussels and Cd concentrations in the seep mussels, the majority of metal concentrations measured in the gill, mantle, and visceral mass of chemosynthetic mussels did not differ greatly from those found in mussels inhabiting coastal environments. Gill, mantle, and visceral mass concentrations of Mn fell within the range of whole body tissue measurements in the coastal mussels *Mytilus edulis* and *M. californianus* (Goldberg et al. 1978). Concentrations of Zn in tissues of vent and seep mussels, with the exception of the gills of vent mussels, fell below O'Connor's (2002) 15th centile value (86 μg/g dry wt.). The mean Cd concentration in the mantle of the vent mussels fell below O'Connor's (2002) 15th centile value (1.2 μg/g dry wt).

Highest metal concentrations in the gill relative to the mantle and visceral mass are consistent with the hypothesis that the gills are the primary route of metal uptake in chemosynthetic mussels. Metal concentrations in coastal bivalves are generally highest in the gills and digestive tissue because uptake primarily occurs via absorption across the gill and in conjunction with food (Laughlin et al. 1986; Langston and Spence 1995). Limited filter feeding in chemosynthetic mussels, due to reliance of these mussels on bacterial symbionts in the gill for nutrition (Cavanaugh 1983, Rau 1985), suggests the gills may be the primary location of metal uptake in chemosynthetic mussels. Smith and Flegal (1989) suggest that the reduced size of the gut in *Bathymodiolus* spp. results in metal concentrations within the digestive tract accounting for < 5% of the metal concentration in the whole body. In *B. thermophilus* from the Galapagos Rift, both gill and digestive tissue were metal enriched relative to other tissues (Smith and Flegal 1989). Lower metal concentrations in the mantle relative to gill and digestive tissue is consistent with metal concentrations measured in *Bathymodiolus* sp. from hydrothermal vent sites on the Mid-Atlantic Ridge (Geret et al. 1998; Rousse et al. 1998). Metal deposition in the byssal thread, resulting in elimination of the metal from the soft tissue, is documented for various metals in zebra mussels, *Dreissena polymorpha* (Gundacker 1999). Concentrations of metals in the byssal thread of *Bathymodiolus* spp. mussels have not yet been quantified; therefore it is unknown what role this plays in metal detoxification in chemosynthetic environments.

Lack of elevated metal concentrations in mussel tissues, including Ca, Mn, Co, Cu, Zn, and Cd, that have previously been shown to be elevated in vent fluids

may be influenced by the speciation and thus bioavailability of metals in the venting fluid. The free-ion activity model suggests that biological responses to metal exposure in marine organisms will be correlated with the free-metal ion activity rather than the total concentration of the metal (Campbell and Tessier 1996). Metal sulfides and other metal-rich deposits, which are not bioavailable forms of metals, are formed rapidly when vent fluid meets seawater (Edmond et al. 1979, Bonatti 1983). Equilibration of metals in cooling vent fluid is achieved at varying rates for different metals (Seewal and Seyfried 1990), affecting the speciation of each metal. If the formation of metal sulfides is sufficiently rapid, metals may not be present in bioavailable forms near the mussels.

In order to investigate whether metals are stored in different forms in mussels from hydrothermal vents, cold seeps, and coastal zones, models that have been shown to predict metal concentrations in coastal mussels were applied to chemosynthetic mussels. Metal concentrations in coastal mussels are frequently correlated with the size of the mussel (Newman and Heagler 1991); however, shell length was not a good predictor of metal concentrations in chemosynthetic mussels (Table 6). There is also no evidence that Ca concentrations in vent and seep mussels are good predictors of metal accumulation in chemosynthetic mussels (Table 7). Lack of significance in linear regressions relating metal concentrations with either Ca concentration (Table 7) or the solubility of the metal phosphate (Table 5) indicates that chemosynthetic mussels do not follow the metal-accumulation kinetics suggested by Jeffree (1988). It is possible that granule-dominated metal storage in chemosynthetic mussels may not coincide with the metal-accumulation patterns predicted by Jeffree (1988). Lower

Ca concentrations in freshwater relative to seawater and correspondingly increased proportions of other metals relative to Ca may make this model more applicable to freshwater than seawater environments. Altered ratios of Ca to other metals in vent fluid relative to ambient seawater may also influence the effectiveness of this model in vent mussels.

Linear regressions predicting metal concentrations in the gill, mantle, and visceral mass of *Bathymodiolus* spp. from vents and seeps based on ion characteristics indicate that χ^2r is a good predictor of metal concentrations in chemosynthetic mussels (Table 5). Softness index also predicted most of the metal concentrations in tissues reasonably well. The absolute value of Log K_{OH} was useful in predicting metal concentrations in seep mussels but not vent mussels. The significant relationship between metal ion characteristics and metal concentration in tissues may be biased by the large difference in the magnitude of Ca and Mg concentrations in the vent environment relative to the concentrations of other metals. In general, linear regressions predicting metal concentrations based on ion characteristics were more significant for seep mussels than vent mussels (Table 5).

Lack of correlations between the concentration of Ca relative to the concentration of other metals and the solubility of metal phosphates relative to their concentrations in chemosynthetic mussels suggest that granules do not solely dominate metal storage in chemosynthetic mussels. Identification of metallothioneins in vent mussels from the Mid-Atlantic Ridge shows that such proteins play a role in metal detoxification in vent mussels (Geret et al. 1998). Significant correlations between metal ion characteristics and their concentration in chemosynthetic mussels,

particularly those from the cold seep, support a model of metal accumulation dominated by affinity for ligands, such as the sulfhydryl groups of metallothioneins. In summary, metal storage in *Bathymodiolus* spp. mussels from chemosynthetic environments may not differ greatly between hydrothermal vents and cold seeps. Metal concentrations in *Bathymodiolus* spp. mussels were similar to metal concentrations in coastal mussels. Since metal concentrations in hydrothermal vent fluids are typically higher than those at cold seeps or in coastal waters, it is possible that many of the metals at the hydrothermal vents are not bioavailable. Preliminary application of bioaccumulation models for coastal models to the metal concentrations found for chemosynthetic mussels in the study suggests that ion characteristics, especially χ^2r and softness index, may be useful in predicting metal concentrations in chemosynthetic mussels.

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